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THE TOXICITY OF COPPER SULFATE TO THE SPORES OF TILLETIA TRITICI (BJERK.) WINTER*

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INTRODUCTION

Copper sulfate has long been recognized as an effective fungicide for the control of *Tilletia tritici* (Bjerk.) Winter. The usual treatment is to dip seed wheat infected by the organism in a copper sulfate solution. That this treatment greatly diminishes the number of diseased plants is certain, but just how the individual spores are affected physiologically by the copper has never been fully determined.

REVIEW OF LITERATURE

According to Evans,[4] "Tessier, 1889, seems to have been the first to use copper compounds for the prevention of smut." In a work published in 1807, Prevost[7] gives a careful account of the effect of copper on the spores of wheat smut, *Tilletia tritici*. Stevens[9] studied the toxicity of a large number of chemical compounds and concluded that all the copper salts agree closely in their toxic action on fungous spores. Duggar[3] made an extensive study of spore germination as

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affected by certain chemical, as well as physical, stimuli. He found that fungi were stimulated very little, if any, by copper sulfate and that they tolerate only very dilute concentrations of copper sulfate. Clark[1, 2] studied the toxicity of copper sulfate to 15 fungi which represented fairly well the natural groups, and found that 12 forms represented a range of lethal concentration of .0168 N to .0099 N, or slightly less than 70 per cent variation. He also found that copper sulfate was much more toxic when dissolved in pure water than when dissolved in any other medium. Hawkins,[5] using distilled water, found that a .00006 N concentration of copper nitrate practically inhibited the growth of *Glomerella cingulata.*

In summing up the work of previous investigators, it is readily seen that the growth of most fungi is inhibited by rather low concentrations of copper compounds.

METHODS

In starting this research, the first problem was to determine the conditions under which maximum uniform germination could be secured.

Stakman[8] writes that rather uncertain and capricious germination was noted by Prevost, De Candolle, Tulsane, Kühn, Fischer von Waldheim, Brefeld, and others. He found that germination required from two to four days in water at room temperature and that all nutrients except soil infusion exerted a harmful effect on germination. McAlpine[6] also germinated the spores in water in two or three days. Wilcox[10] was not able to obtain more than 8 to 10 per cent germination in distilled water, and that only after a period of twenty-five to thirty days.

Culture solutions.—In preliminary experiments to determine the best medium for germination the following solutions were used:

No. 1. Water extract from Yolo sandy loam soil.
No. 2. Same as No. 1 diluted to one-half strength.
No. 3. Same as No. 1 diluted to one-quarter strength.
No. 4. Water extract of San Joaquin sandy loam soil.
No. 5. Same as No. 4 diluted to one-half strength.
No. 6. Same as No. 4 diluted to one-quarter strength.
No. 7. Distilled water.
No. 8. Tap water.

Extracts of soil were made by mixing one volume of soil with two volumes of water and autoclaving for one and one-half hours at 17 pounds pressure. The liquid was filtered off under pressure and sterilized.

Solution No. 5 gave the highest per cent and most uniform germination and was therefore used for the experiment.

Using Baker's analyzed copper sulfate, a .1N stock solution was made up. The concentrations required for this experiment were then made up by a series of dilutions and were prepared without the measurement of less than 10 c.c. in any case. Standard pipettes and volumetric flasks were used.

Temperature.—In preliminary experiments to determine the optimum temperature for germination, tests were made at room temperatures and controlled temperatures, 48° F., 53° F., 58° F., and 63° F. The latter temperatures were maintained by an automatically regulated chamber placed in a 40° F. cold storage room. Based on the results of the above experiments, it was decided to conduct one set of experiments at a controlled temperature of 58° F. and one set at room temperature which varied from 56° to 62° F.

Method of culture.—Two methods of culture were used: (*a*) the sealed hanging drop method as described by Clark[1] and Duggar[3]; and (*b*) the ventilated hanging drop. The latter was prepared by supporting the cover glass on two strips of paraffin 4 mm. square and 25 mm. long. By heating the slides the strips were sealed to them, and the cover glasses were made fast by pressing the edge with a hot needle. These cultures were kept in a moist chamber, in order to keep the drop from evaporating.

Examination of cultures.—Cultures were examined on the fourth day, and each day thereafter until they were nine or ten days old. Counts of ten spores were made from each of five different fields and the average per cent of germination recorded.

DATA AND DISCUSSION

The data showed that there was little, if any, difference between the germination at the laboratory temperature and that at the controlled temperature. Likewise the germination in the sealed hanging-drop cultures was not essentially different from that in the ventilated hanging-drop cultures.

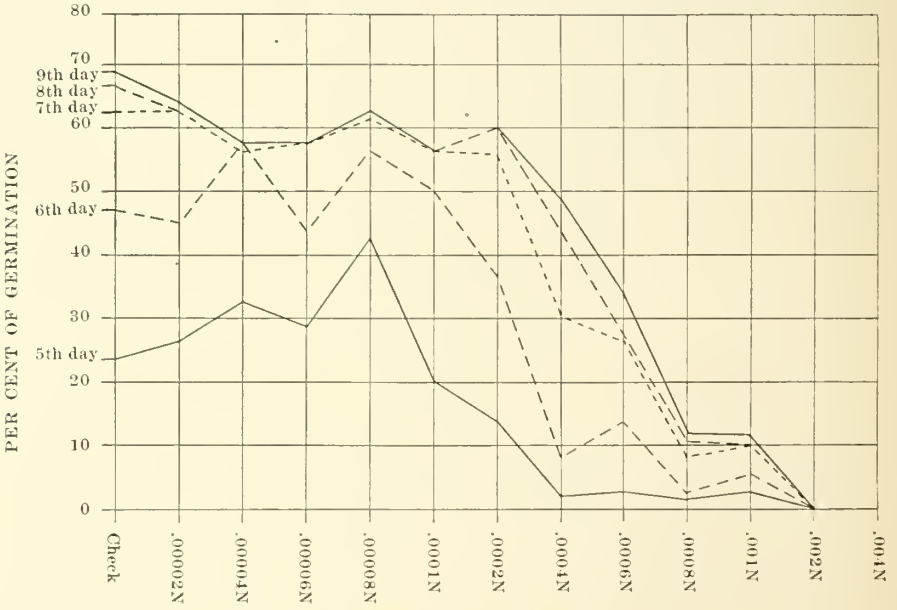

Fig. 1. Concentration of copper sulfate.

Under all conditions germination was somewhat erratic. Frequently duplicate mounts showed variations of 30 to 50 per cent in germination, but this was probably due, to some extent, to contamination by moulds, which frequently occurred toward the end of the incubation period. The technic followed in making up the mounts undoubtedly was responsible for some of the erratic germination. A small drop spread in a thin layer gave better germination than a large drop. The number of spores in proportion to the amount of solution was probably another factor. In a given drop the amount of copper for each spore would decrease as the number of spores increased. So, with a few spores, there might be a decided decrease in germination, while with a large number of spores there would be little decrease.

Since there was so little difference in germination under the different conditions of this experiment, the results were averaged together and are presented in a graph (fig. 1).

It will be noted from the data represented in the graph that an .002N solution of copper sulfate was sufficient to inhibit the growth of the spores of *Tilletia tritici* under the condition of this experiment. Cultures of .002N to .008N were kept for 20 days without any germination. A soil extract of a different concentration probably would have given a different point of inhibition. Clark[2] found the lethal concentration of copper sulfate to be .0076N when a beet decoction of normal strength was used; while it was .0034N, or approximately one-half when the decoction was diluted to four volumes.

Concentrations of .0008N and .001N caused a decided decrease in percentage of germination. The promycelia were very short and distorted, in many cases never reaching a length greater than 20 to 30μ. No sporidia were found in cultures of this concentration, and because of their weakened condition it is very doubtful if any of these spores would be capable of infecting a wheat plant.

The concentration of .0006N copper sulfate caused many signs of abnormal germination, but frequently a spore would germinate in a perfectly normal manner, in so far as one could determine from a superficial examination. In the more dilute copper solutions, germination apparently was normal with no depression of any very great consequence in the percentage of germination, while in the more concentrated copper solutions there was some delay in germination and a very marked decrease until the point of inhibition was reached.

CONCLUSIONS

Under the conditions described the following conclusions may be drawn:

1. In a culture solution consisting of a water extract of San Joaquin sandy loam soil, a .002N concentration of copper sulfate is sufficient to inhibit the germination of spores of *Tilletia tritici*.

2. In concentrations of .0008N and .001N there was very little germination and that which occurred was decidedly abnormal in character, the promycelium being very short and distorted.

3. It is doubtful if the abnormal promycelium obtained in concentrations of .0008N and .001N was capable of causing any infection.

4. Germination of spores in a .0006N copper sulfate solution was abnormal except for occasional ones which seemed to develop in the normal way.

5. In the more dilute copper sulfate solution, .00002N to .0004N, germination apparently was normal. Occasional spores in concentrations of .0004N and .0006N showed some copper injury.

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